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Reproduction and Ontogeny of *Gomphocythere australica* Hussainy (Cytheridae, Ostracoda)

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Summary: The reproduction and ontogeny of *Gomphocythere australica* were studied. The species is dioecious, with a sex ratio of 1: 1. Reproduction is by syngamy only. Eggs are retained in the posterior end of the female carapace, only larvae of 2nd stage being released. There are nine developmental stages; the sequence of addition of appendages at each moult follows that described by Müller. The furca, however, becomes rudimentary at the fifth instar and in the adult is represented by two thin, juxtaposed lamellae. Appendages are added at each instar except the third.

INTRODUCTION

Most published work on the life history and metamorphosis of Ostracoda appeared in the latter half of the nineteenth century and in the first two decades of this century; Kesling (1951) reviewed it. He also gave a detailed description of the morphology of the various moult stages of *Cypridopsis vidua* (O. F. Müller 1776) Brady 1867. More recently Theisen (1966) described life histories of seven species of Danish brackish water Ostracoda. Most studies seem to have been carried out on cyprid ostracods. Comparatively little is known of the development of freshwater Cytheridae.

The present paper records some observations on reproduction and compares the various instars during post-embryonic development of a cytherid, *Gomphocythere australica* Hussainy 1969.

MATERIALS AND METHODS

Quantitative samples of sediments were collected from the littoral regions of Lake Purrumbete, Victoria, Australia, during July-September 1967. In the laboratory, samples were transferred to enamel trays and aerated. De-ionized water was used to make up the loss due to evaporation. Specimens appeared after about two or three days. The data obtained from field-collected material was supplemented with data from laboratory-cultured material. Cultures were maintained at 20°C ± 2°C in 'cavity blocks' using water and detritus from the culture trays.

Most of the observations on the development were based on live material; however, material preserved in 70% alcohol was also used. For microscopic examination specimens were mounted in polyvinyl alcohol with chlorazol black. All sketches were made using a camera lucida.

DISCUSSION

Ostracoda are dioecious and both parthenogenetic and syngamic reproduction is common among them. It appears that an alternation of parthenogenesis and syngamy does not occur (Ferguson 1944, Kesling 1951). With the known exception of *Darwinula stevensoni* Brady and Robertson (1870) many Myodocopida and several cytherid genera including *Gomphocythere*, all are oviparous (McGregor 1968, McKenzie and Hussainy 1968). Some species reproduce by parthenogenesis only; in *Cypridopsis vidua*, for example, the occurrence of males has never been established (Kesling 1956).

Males have been recorded for all species of *Gomphocythere*, and there is a distinct sexual dimorphism. Reproduction is by syngamy. Females are much larger than males and their shell is very swollen at its posterior region to form a roomy 'incubatory cavity' for the reception of ripe ova. The sex ratio appears to be almost 1:1 (2330 ♂ : 2010 ♀, during July-September 1967).

Copulation

Copulation in Ostracoda has been observed in several species, e.g. *Entocythere heterodonta* Rioja (Kaufman 1892) and *Potamocypris smaragdina* Vavra 1891 (Ferguson 1944). In the present in-

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vestigation, copulation was observed several times in the laboratory cultures and in field-collected material. The male attached itself to the dorso-posterior region of the female shell and held securely thereto by means of its second antenna. The ventral cavity of the male was kept open by the long curved claw of the 2nd thoracic appendage which was used as a wedge. The copulatory organ of the male was rotated and then inserted between the valves of the female shell.

Broodcare

Among Cytheridae, broodcare is known or suspected for several marine or euryhaline genera, but *G. australica* Hussainy 1969 is the first species among freshwater Cytheridae for which it has been recorded (McKenzie and Hussainy 1968).

Larvae hatch out and undergo two moults while they are still within the female carapace. Only 2nd instar larvae are released. *Gomphocythere australica* may be unique among freshwater forms in being the only freswater representative which retains the young through embryological development to the 2nd instar. *Darwinula stevensoni* and other species of *Darwinula* are known to care for their young through embryological development to the first instar only (McGregor 1968).

Two hundred and fifty females were dissected and examined for the broods. All except six were ovigerous and carried either eggs or juveniles or both. No females carried juveniles older than 2nd larval stage. The total number of eggs plus juveniles brooded per female varied from 1-20 but 14-18 was very common.

Broods from 25 females were isolated separately in 'cavity blocks'. They yielded 173 eggs, 78 first instar larvae, and 16 second instar larvae. These were allowed to develop in water and sediments from the culture tray. One hundred from 173 eggs hatched out into first instars. Of these, 50 moulted to 2nd larval instars. Only 20 moulted to 3rd instar, and 15 to 4th instar larvae. No development took place in the laboratory beyond the 4th larval stage. These observations are adequate enough to infer that eggs and early instars are capable of development even outside the incubatory chamber. As the first larvae are stationary in habit broodcare could be an adaptation to ensure protection and better chance of survival of juveniles.

In another experiment, 50 ovigerous females were isolated to observe the period of larval release. Water and organic detritus were provided from the culture tray. It was observed that larvae were released for about six weeks. Almost all the instars released (600) were the second larval stage. During this period only ten eggs and five first stage instars were seen in the subculture. These could have been released accidentally or from two specimens which died during experimentation.

Process of Moulting

Relatively little is known about the process of moulting. Wolgemuth (1914) from his studies on *Cyprinotus incongruens* Ramdohr 1808 believed that the organism sheds its antennules first, then the other appendages in order towards the rear. Schreiber (1922) observed the moulting of *Eucypris virens* (Jurine) 1820 and concluded that rear appendages were the first to be freed from the old skeleton and antennules were the last. The process may vary with different species.

In the present studies complete moulting of two 2nd instar juveniles was observed. The moulting took about two hours. It was accompanied by barely perceptible movements with prolonged intervals in which the animal is apparently motionless and lying flat on its side. The branchial plates were in normal motion. The valves were slowly spread open to an abnormal width. The maximum opening of the valves at the anterior end was 96μ and 50μ at the posterior. The first breaking of the exoskeleton took place with the opening of the left valve. This was followed by the replacement of the right valve. After about one hour, the body wall was separated from the lining of the valves in the dorsal part of the head region. Antennules were then withdrawn slowly from their sheaths. The antennae followed this sequence and then the other appendages. The shell in the posterior region was freed using the antenna, and the organism crawled out of the old skeleton. It lay motionless for some time, presumably for the hardening of the raw skeleton, and moved again with the help of the antenna.

Ontogeny

Claus (1868) working on *Cypris ovum* Jurine and *C. fasciata* Müller made the first extensive study on the ontogeny of ostracods. Later the morphology of various instars of other species have been described by Müller (1894), Schreiber (1922), Scheerer-Ostermeyer (1940) and Kesling (1951). At first sight, their reports seem to show a great variation; but, a detailed comparison reveals that the disparity arises not from the observations of number of appendages in each instar, but from the interpretation of appendages in juveniles. The appendage which Müller referred to as an incipient furca has been described by Claus as second leg. Thus the apparent order of appearance of the various appendages differs considerably according to different authors, although their observations are in agreement.

Schreiber (1922) and Scheerer-Ostermeyer

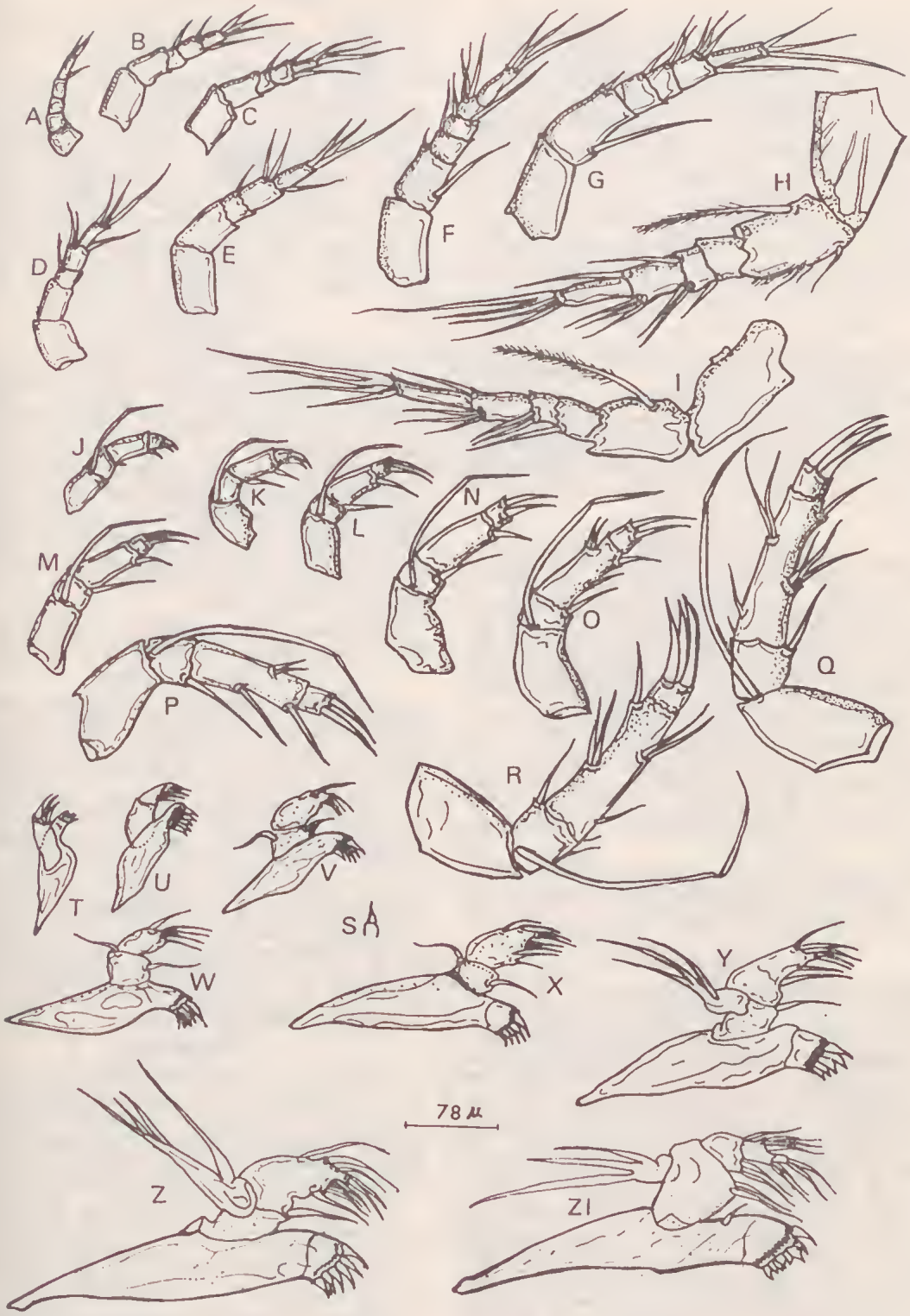

FIG. 1—A-I Antennules of instars 1-9. J-R Antennae of instars 1-9. S-Z_1 Mandibles of instars 1-9.

(1940) followed Müller (op. cit.) in their studies on ostracod ontogeny. It is seen that the number of appendages for each instar is the same in both systems followed by Claus and Müller. In Müller's system the furca appears early and the body appendages are added in orderly sequence from the anterior to posterior. This system seems to be a widely accepted one (Kesling 1951). In *Gomphocythere australica* the sequence of the addition of appendages at each moult followed that described by Müller. The furca, however, became rudimentary at 5th larval stage and in the adult is represented as two thin juxtoposed lamellae.

Egg

Eggs are grey-white, nearly spherical, opaque, and approximately 90μ in diameter. As development progresses they turn translucent. There is a soft single-walled, white opaque shell. The opaque nature of the shell makes it impossible to observe the egg contents. When the larvae hatch, a split occurs dividing the shell into two equal halves, which usually remain attached.

First Instar

Carapace elliptical, 162μ in length and 112μ in width; soft and weakly sclerotized and transparent. Eye is large. The instar has three pairs of appendages; antennule, antennae and mandible (Fig. 1A, J, S). Antennule with four podomere and only three setae, two of which are on the terminal podomere. The second podomere with spine at its joint with the terminal podomere. The antenna with four podomeres with two setae on the terminal podomere and one on the subterminal podomere. The basal podomere with a two-jointed spine. Mandible short, stump-like, comprises two short podomere set on an elongate extension of the body, with a strong seta at the terminal end.

The instar is stationary and antennules and antennae are utilized for catching food. Duration 14-18 days. Generally, moulting takes place on the 16th day.

Second Instar

Larvae are released as 2nd instars only. It is very difficult to observe the detailed process of the laying of larvae because of the opacity of the shell. However, observations suggest that the ovigerous female stands motionless in the organic detritus with the valves partially opened at the beginning of the process. They are released singly. It appears that the 2nd and 3rd legs assist in releasing the larvae.

Description as follows: length 192μ, height 124μ and width 109μ. When newly moulted it is transparent. Shell slightly enlarged but the rim still very simple. Muscle scars clearly seen. Eye is approximately the same size as in the first instar and still located near the middle of the shell. Antennule (Fig. 1B) composed of four distinct podomeres. Antenna (Fig. 1K) consists of four podomeres, the terminal podomere has two setae, each of the podomeres with one seta each. Mandible (Fig. 1T) greatly altered from its structure of the first instar. The terminal podomere is completely set off and equipped with teeth at its distal end; in addition it has a palp of two distinct podomeres.

'Anlagen' of two new appendages are added; these are interpreted as the primordia of maxilla and furca. The maxilla is swollen with a pointed projection at its anterior ventral side. In the dorsal posterior area a slight lobe is seen which may be interpreted as the 'anlagen' of the exopodite plate. The furca is represented by a strong stump-like projection of the body with a long terminal seta. The mouth has shifted to the middle of the body. The duration of this stage is 12-17 days but 14 days is quite frequent.

Third Instar

During this stage no new 'anlagen' are added, a further development of the already present appendages takes place, length 224μ, width 135μ and 120μ in height. Antennule (Fig. 1C) consists of the same number of podomeres as in the previous instar. Antenna (Fig. 1L) has the same form and essentially unchanged. Mandible stronger and slightly elongated (Fig. 1U). Terminal end of the maxilla (Fig. 2A) divided into 'masticatory' processes but without a palp. Expodite bears six setae. Furca, basically the same, a stump of the body with a long seta. Mouth still in a median position.

The duration of this instar is from 20-26 days. Generally moulting takes place on the 24th day.

Fourth Instar

Length 259μ, width 160μ and height 120μ. Antenna has five podomeres (Fig. 1M), with marked increase in length. It still maintains the same approximate form. The second podomere of the protopodite of mandible (Fig. 1V) separated from the basal podomere, but the general form remains the same. The maxilla (Fig. 2B) approaches a definite form with an increase in the number of setae on the respiratory plate. There is a definite 'anlagen' of the first thoracic leg, a roughly triangular plate with a ventral lobe hanging free with a short claw (Fig. 2H). Furca basically remains the same in shape and size.

The fourth instars survived for 20 days but did not moult. The description of the subsequent instars is based on field-collected material.

Fifth Instar

Length from 312 to 320μ. Shell thicker and with more complex rim. The colouration very similar to that of adult (brownish). The antennule (Fig. 1E) consists of the same number of podomeres. The antenna (Fig. 1N) increased in length, the mandible (Fig. 1W) well built. The palp of the maxilla (Fig. 1C) divided into four podomeres, the respiratory plate larger and equipped with thirteen setae. The first thoracic leg (2I) three-segmented with a claw at the distal end. The basal segment is equipped with a pair of setae.

'Anlagen' of second thoracic leg (2N) developed as an elongated, backwardly-directed process of body terminating in very blunt clawlike process. Attach-

FIG. 2—A-G Maxillae of instars 3-9. H-M 1st thoracic leg of instars 4-9. N-R 2nd thoracic leg of instars 5-9. S-V 3rd thoracic leg of instars 6-9.

ment immediately behind the first thoracic leg. The furca is reduced and is represented by a short projection of the body, a short spine.

Sixth Instar

All appendages are present for the first time in the sixth instar. The remaining ontogenetic stages show further development of the appendages and the 'anlagen' and the completion of the sex organs.

Sixth instar length 368μ antennule (Fig. 1F) with six podomeres, with well-developed spines and setae. Antenna (Fig. 1O) with four podomeres. Claws well-developed and chitinized. Mandible (Fig. 1X) slightly longer. Maxilla (2D) also reached its definitive form; in this instar the number of setae are also increased. First thoracic leg (2J) fundamentally remained the same, except for a slight increase in size. Second thoracic leg (Fig. 2O) four-segmented with the terminal segment pointed to form a claw.

The anlage of the third thoracic leg (2S) is an elongate, backwardly directed process of the body with a stubby chitin at the end. It resembles the anlage of the second thoracic leg in the fifth instar.

Seventh Instar

Length 448μ to 480μ, all appendages well-defined and the anlagen of the sex organ. The antennule (Fig. 1G) consists of six podomeres. The antenna (Fig. 1P) approached the definitive form with well-developed claws. Mandible (Fig. 1Y) increased in size with an exopodite with three setae. Maxilla (Fig. 2E) greatly enlarged. Exopodite plate (branchial plate) much larger and equipped with a greater number of setae. First two (Fig. 2K, R) pairs of thoracic legs five-segmented, well built and of definitive form. Terminal segment pointed to form a claw.

The structure of the furcal rami remains the same, as in the previous segment. There is no development of genital lobes and the external genitalia.

Eighth Instar

The eighth instar resembles the adult very closely except for size and sex dimporphism. It possesses all the appendages in the definitive form. The genital lobe makes its appearance as a rudiment. The appendages have no marked change from the previous instar. This instar measures 520μ to 592μ in length.

Ninth Instar (*adult form*)

The adult differs from the eighth instar in the external chitinous genital structure and the completion of sex organs in both sexes. The appendages are strongly chitinized, eye is median, valves of the shell are dark.

There is a distinct sex-dimporhism both in size and shape of the individuals. The lengths of male and female are 700μ and 860μ respectively. Caudal rami are very different, forming juxtoposed thin lamellae curving anteriorly, with a plumose seta at their base. Posterior end of each lamella is divided into three successive short 'liquiform lobules' clothed at the tip with long diverging cilia.

The copulatory appendage of the male is very massive, terminating in a quadrangular plate.

Fig. 3—A. Furca of female. B. Penis.

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